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Comparative analyses of alterations in carbohydrates, amino acids, phenols and lignin in roots of three cultivars of *Xanthosoma sagittifolium* infected by *Pythium myriotylum*

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The putative role of carbohydrates, amino acids, phenols and lignin in the susceptibility of *Xanthosoma sagittifolium* to *Pythium myriotylum* root rot disease was investigated by comparing the alterations of these groups of molecules in the roots of the white (susceptible), red (tolerant) and yellow (resistant) cultivars. At Day 8 after inoculation, total soluble sugars decreased. There was a positive correlation between this decrease and the root rot disease index for the white, the red and the yellow cultivars. At Day 2 proline was no longer detected in the yellow cultivar, while its content was 83% and 84% of the initial values in the white and red cultivars, respectively. At the same time, total amino acid content increased in the three cultivars. However, this increase was negatively correlated to the disease only in the yellow cultivar (r_p = 0.940, P < 0.05). The amounts of phenols and lignin increased in the three cultivars. The increase in phenols was correlated to the disease (r_p = 0.951 and 0.890; P < 0.05) for the red and yellow cultivars, respectively. Thin layer chromatography revealed five amino acids (arginine, proline, glycine, alanine and tyrosine) in the healthy roots of the white and the red cultivars, and three amino acids (arginine, glycine and alanine) in the yellow cultivar. Following inoculation, glycine and flavanol-like phenols (λ_{max} = 220nm) were detected in the three cultivars. The content of a non-categorised group of phenols (λ_{max} = 270nm) detected in all cultivars increased after inoculation in the red and yellow cultivars. Phenols of the hydroxycinnamic group (λ_{max} = 350nm) were specific to the inoculated roots of the red and the yellow cultivars. Glycine and flavanol-like phenols thus seem to have accumulated in *X. sagittifolium* roots after inoculation by *P. myriotylum*, and phenols of the hydroxycinnamic group seem to be specific to the red (tolerant) and the yellow (resistant) cultivars.

Introduction

Several defence mechanisms are known to be induced in plants challenged by pathogens (Lyon *et al.* 1995). Such mechanisms culminate in a number of biochemical and physical changes, including a decrease in soluble carbohydrate content (Jeun and Hwang 1991), accumulation of amino acids (Nemec 1995), lignification and suberisation of the plant cell wall (Stein *et al.* 1993), deposition of callose (Brown *et al.* 1998), *de novo* synthesis of pathogenesis-related proteins (Van Loon and Van Strien 1999) and biosynthesis and accumulation of secondary metabolites such as phenolics (Bennet and Wallsgrove 1994).

The implication of soluble carbohydrates in plant resistance is still unclear. Horsfall and Dimond (1957) suggested that the sugar content of plant tissues could influence their susceptibility to diseases. However, during the infection of other plants, it was found that sugars inhibited or had little or no effect on the development of fungi (Lukens 1970, Gibbs and Wilcoxson 1972).

Increases in amino acids and proteins are ubiquitous responses of plants to pathogen invasion (Van Andel 1996, Van Loon and Van Strien 1999). Amino acids may be involved in the host metabolism associated with disease resistance. Tyuterev and Tarlakovskii (1994) and Starrat and Lazarovits (1996) have reported that the glutamine, glycine, histidine and arginine content of the resistant genotypes of wheat and tomato are higher than that of susceptible ones. Thus, there may be possible correlation between the accumulation of amino acids and resistance to pathogens. Accumulation of proline is a widespread plant response to environmental stress (Verslues and Sharp 1999, Sanchez *et al.* 2002). Because of its zwitterionic and highly hydrophilic characteristics, proline can accumulate at high concentrations in the cytosol without interaction with cellular structures or metabolism; it then can act as an osmoticum (Verslues and Sharp 1999). Beside this osmotic role, proline protects enzymes against the deleterious effects of various

stresses and also acts as a storage compound for reduced C and N, or as a scavenger of hydroxyl radicals (Delauney and Verma 1993).

Phenolic acid intermediates in phenylpropanoid metabolism play various important roles in plant defence systems. They are precursors for the synthesis of lignin (Lewis and Yamamoto 1990) and phenylpropanoid phytoalexins (Kessmann *et al.* 1990, El Hadrami 2002) and their deposition into the cell walls after pathogen infection is an important defence reaction. Their accumulation can block the growth/development of fungi (Assabgui *et al*. 1993). Plant quinones and phenylpropanoid phytoalexins have an antibiotic property and are derived from phenolic compounds and aromatic amino acids (phenylalanine and tyrosine) through oxidation. Generally, the amount of phenolic compounds increases in plants after infection (McLusky *et al.* 1999). However, Jeun and Hwang (1991) have shown that the amounts of total phenolics in pepper stems are relatively low in the resistant cultivar Kingkum.

Lignins are complex polymers of phenylpropanoids mainly localised in the secondary cell walls and middle fibres of vascular plants (Lewis and Yamamoto 1990). In higher plants, lignin is deposited in wounded or infected boundary zones of normally unlignified tissues, where they act as a protective barrier to pathogen ingress and/or against moisture loss, due to their metabolic inertness and hydrophobic nature (Boudet 1998). For example, in banana roots, the presence of lignified and suberised layers in endodermal cells contributes to limit invasion of the vascular bundle by the nematode *Rodopholus similis* (Valette *et al.* 1998).

The corm of *X. sagittifolium* (cocoyam) provides food energy, vitamins and family income to millions of people in the tropics and subtropics. Worldwide production of cocoyam is Africa 60%, Asia 32% and the Caribbean 8%, for a total of about eight million metric tons per annum (Onokpise *et al.* 1992). The main constraint of cocoyam production in Cameroon is the root rot disease caused by *P. myriotylum* (Xu *et al.* 1995). This parasite can cause reductions in production as high as 90% in some farms in Cameroon (Schafer 1999). Such a severe decline has stimulated research towards the development of root rot disease resistant cultivars. The breeding of varieties resistant to root rot disease by classical and/or biotechnological methods has, as a prerequisite, the characterisation of structural, biochemical and molecular events involved in the resistance mechanism of this species. In this paper we analyse the possible involvement of amino acids, sugars, phenols and lignin in the defence mechanism of cocoyam against *P. myriotylum*. The changes in the levels of these metabolites among three cultivars available in Cameroon are discussed. The three cultivars differ in their resistance to root rot: namely, the white, pink/red, and yellow cultivars are categorised as susceptible, tolerant and resistant, respectively (Tambong *et al*. 1997).

Material and Methods

Biological materials

In vitro tissue-culture-derived cocoyam plantlets were cultured from apices in the Murashige and Skoog (1962) mineral medium, according to the method of Omokolo *et al.* (1995b). Plantlets were acclimatised in a mixture of soil/sawdust (1:2). *P. myriotylum* isolates were obtained from diseased cocoyam roots harvested from the Yaoundé area and purified according to Xu *et al.* (1995). For each experiment, 20 acclimatised plantlets were inoculated with a calibrated suspension of *P. myriotylum* zoospores (10^6 ml^{-1}) (Pacumbaba *et al.* 1994) and left to acclimatise (Omokolo *et al.* 2001). Inoculated and non-inoculated roots were randomly collected every two days until Day 8; they were rinsed under tap water, blotted dry, weighed and the root rot index was determined according to Nzietchueng (1983):

$$\text{root rot index (RRI)} = 4 - P / 25$$

where P = percentage of healthy roots.

Microscopic observations and extractions

Two and eight days after inoculation, unlignified root tips were excised, rinsed in distilled water and directly observed under a light microscope (Zeiss, Axioscope MC-100). Root tissue for extraction was excised 0.5cm beyond the area of necrosis. Where there was no necrosis, tissue samples were obtained at an equivalent location. Amino acids and sugars were extracted under reflux according to Singh *et al.* (1990). One gram of biological material was ground in 8ml of 80% ethanol, and then heated under reflux for 30min. The alcoholic extract was collected after filtration through Whatman No. 1 paper. The residue was collected and re-extracted once. Both extracts were mixed and constituted the crude extract for amino acid and soluble sugar analyses.

Total soluble sugar and amino acid analysis

Total soluble sugar contents were determined using the anthron method of Yemm and Willis (1954): 1ml of the extract was incubated with 5ml anthron solution (0.12g anthron in 100ml 6.5M H_2SO_4) at 90°C for 10min. The absorbance of the green product was measured spectrophotometrically at 630nm. Glucose equivalents were calculated from a standard curve obtained with pure analytical grade glucose.

Total amino acid content was determined spectrophotometrically at 570nm by the ninhydrin method (Yemm and Coking 1955), while proline was determined colorimetrically at 515nm according to Bates *et al.* (1973). Pure analytical grade glycine and proline was used for the standard curves, respectively. Values are expressed in µg (equivalent glycine) per mg for total amino acid and µg (equivalent proline) per g of fresh weight (fwt) of sample. The qualitative analysis of amino acids was done by thin layer chromatography on silica gel. The development solvent consisted of a mixture of butanol, acetic acid and water (4:1:1; v/v/v), and proline, arginine, phenylalanine, glycine, alanine, tyrosine and lysine were used as references. For each sample, a volume of extract containing 4µg of amino acids was deposited on the silica gel at 1cm from the bottom of the plate (10cm x 21cm). The plate was placed in the development tank and the mobile phase allowed to rise to 1cm from the top of the plate. Thereafter, the chromatograms were air-dried, the amino acids revealed *in situ* by spraying a solution of 1% ninhydrin (w/v) on the plates, followed by heating at

110°C for 10min. Amino acids were identified by comparing their Rf values with the references.

Phenolic analysis

Phenolic compounds were extracted using 0.1N HCl. One gram of fresh roots was ground in 3ml HCl. After 30min incubation at room temperature, the ground material was centrifuged at 6 000g for 30min. The supernatant was decanted and the precipitate re-suspended in 3ml 0.1N HCl and incubated at room temperature for 15min. After centrifugation the supernatant was collected and mixed with the first to constitute the phenolic extract. The concentration of phenolic compounds was determined spectrophotometrically at 715nm, according to Marigo (1973), using the Folin-Ciocalteu reagent.

For qualitative analysis, phenols were extracted according to the method described by Omokolo *et al.* (1995a), with some modifications. Four grams of fresh root were extracted twice with 50ml of 100% methanol at room temperature for 45min with constant shaking. The methanolic extracts were filtered through Whatman No. 1 paper and the methanol evaporated at 40°C under vacuum, using a rotary evaporator. The pigments were removed from the aqueous phase by the addition of 50ml of 40% $(NH_4)_2SO_4$, 1.4ml of 85% H_3PO_4 and 50ml of hexane. This process was repeated three times. The hexane phase was discarded and the aqueous phases combined and extracted three times with ethyl acetate. The aqueous phase was discarded and the organic phases combined, dried by addition of 5g $MgSO_4$ and filtered after 5min through Whatman No. 1 paper. The salt residue was discarded and the clear organic phase was dried at 40°C under vacuum, using a rotary evaporator. The dried extract of soluble phenolic compounds was dissolved in 3ml of pure methanol. For analysis, extracts were diluted 600 times and the absorbance scanned between 200nm and 450nm. Standard spectra were obtained with pure analytical grade catechin (λ_{max} = 220nm) and ferulic acid (λ_{max} = 350nm).

Lignin was extracted according to the two-step method described by Rama Devi and Prassad (1996). In the first step, the ground plant material was incubated in water at 100°C for 30min and filtered. The filtrate was later treated with a solution of 3% sodium dodecyl sulphate at 120°C for 60min, placed in 85% ethanol (75°C) for 30min and lastly in acetone (56°C) for 30min. During the second step, the filtrate was dried overnight at 30°C. Ten milligrams of the dried material was digested in 2.5ml acetyl bromide for 30min at 70°C on dry heat, and 0.25ml 7.5M hydroxylamine hydrochloride was added. After cooling in an ice bath, the volume was adjusted to 15ml with a mixture of 2M NaOH:glacial acetic acid (1:5; v/v). After incubation for 2h at room temperature, the lignin was quantified spectrophotometrically at 280nm, against a control without the dried filtrate. The amount of lignin was expressed as percentage of final dry mass (dwt), according to the formula proposed by Rama Devi and Prassad (1996):

$$\text{Lignin (g)} = OD_{280} \times 100 / (\mu \times m)$$

where m = final mass of dry sample (g); μ = 24 = molecular extinction coefficient of lignin.

Statistical analyses

All experiments were repeated at least three times. Data presented here are the means ± SE of at least three independent experiments. An ANOVA comparison between control and infected roots was done at Days 0, 2, 4, 6 and 8 after infection, using the Tukey HSD multiple range tests of the SPSS statistical package, Release 11.0 for Windows. To test if the distribution of the metabolites had any bearing on disease development, Pearson's rank correlation coefficient (r_p) test was performed on root rot index (RRI) and metabolite content at Days 0, 4 and 8.

Results

Root rotting

Figure 1 shows the root rot disease index on Days 0, 2, 4, 6 and 8 for the white, red and yellow cultivars; at Day 4 the index was 1.0, 0.6 and 0.1, and at Day 8 it was 2.6, 1.3 and 0.1, respectively. Fifteen days following plant inoculation with a zoospore suspension of *P. myriotylum*, young roots were completely destroyed while mature lignified root tissues were partially affected (Figure 2B). Microscopic observations of the white cultivar revealed that the pathogen development was localised in the tip (Figure 2C), causing rotting. The destruction of the roots was effective in the root cap four days after infection (Figure 2E). In the yellow cultivar, the fungal colonisation was limited to the external cell layers of the root (Figure 2D, F). In our experimental conditions, leaf chlorosis appeared 10 days after inoculation of the white cultivar. These symptoms were less pronounced in the red cultivar and absent in the yellow one (data not shown).

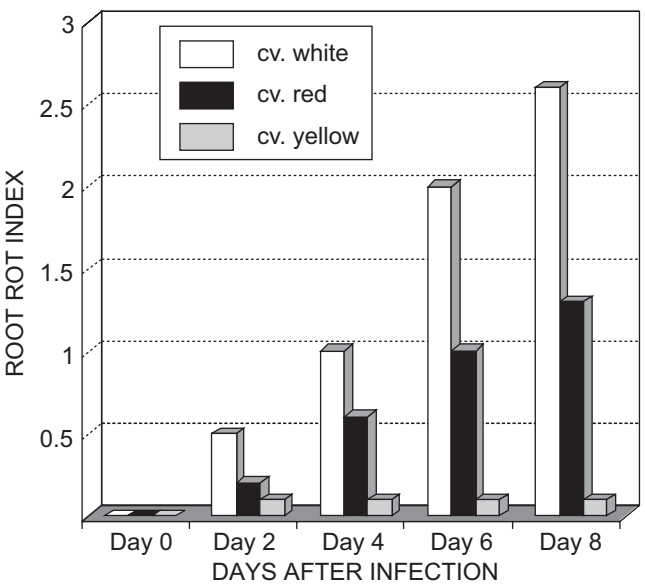

Figure 1: Root rot index in *Xanthosoma sagittifolium* at Days 0, 2, 4, 6 and 8 after inoculation with zoospore suspensions (10^6 ml^{-1}) of *Pythium myriotylum*

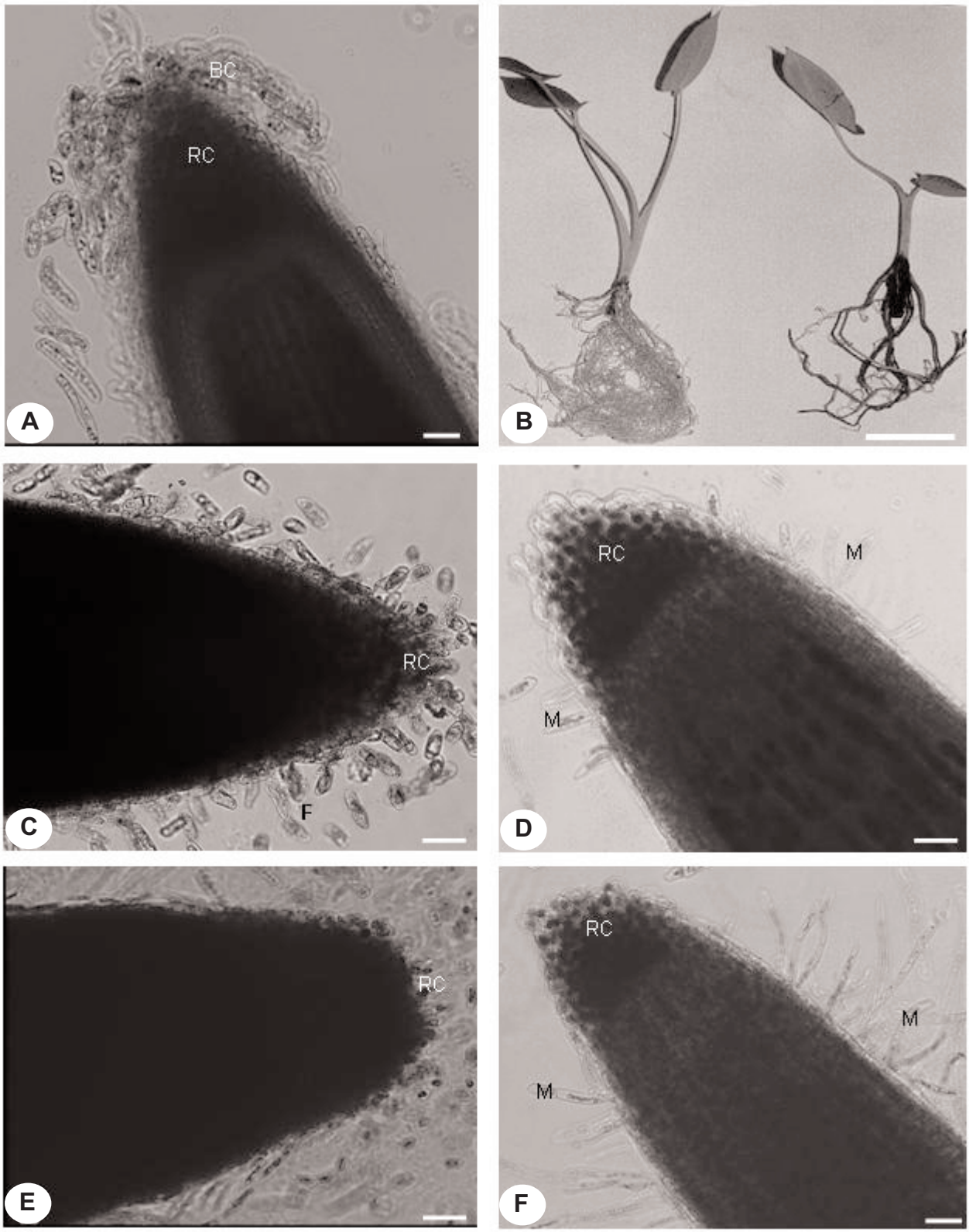

Figure 2: Root colonisation and root rotting in *Xanthosoma sagittifolium* inoculated with zoospore suspensions of *P. myriotylum*: (A) healthy young root showing border cells (BC) and root cap (RC); (B) healthy plant (left) and inoculated plant (right) at Day 8; (C) & (E) light micrographs of inoculated root of the white cultivar showing proliferation of mycelium (M) at the root tip and destruction of the root cap; (D) & (F) the yellow cultivar at Day 2 (D) and at Day 4 (F), showing the localisation of fungal colonisation to the external cell layers of the root. Scale bars: 50µm (A, C, D, E, F); 5µm (B)

Changes in metabolite concentrations

The results presented in Table 1 show that plant inoculation was followed by an alteration of the level of the measured metabolites in the roots. Analysis of variance was used to test for the effect of the inoculation on the distribution of carbohydrates, total amino acids, proline, total phenols and lignin in the root. At Day 8, comparisons were done between cultivars of healthy and inoculated plants (Table 2). Pearson correlation coefficients between metabolites and root rot index were calculated at Days 0, 4 and 8 (Table 3).

Soluble sugar and amino acids

Healthy roots contained 3.2µg, 10.1µg and 10.5µg glucose g^{-1} fresh weight of total soluble sugar in the white, red and yellow cultivars, respectively (Table 1). These levels did not change significantly with aging. Plant inoculation was followed by a significant drop in soluble carbohydrate content. In control plants at Day 8, it represented at $P < 0.001$, 42%, 28% and 33% in the white, the red and the yellow cultivars, respectively. When cultivars were compared among themselves there were significant differences only between healthy ($P < 0.001$) and infected ($P << 0.001$) plants of the white and red cultivars (Table 2). A significant positive correlation was found between the level of sugar and root rot index in all the cultivars (Table 3).

The healthy roots of the white, red and yellow cultivars of *X. sagittifolium* contained an average of 1.8µg, 2.5µg and 1.4µg glycine mg^{-1} fwt total amino acids, respectively (Table 1). These amounts remained constant throughout the experiment. However, when the plantlets were inoculated, the amino acid content at Day 2 increased in the white, the red and the yellow cultivars, by 256%, 225% and 233% ($P < 0.001$), respectively (Table 1). Beyond this age, the amino acid content decreased in the white and red cultivars although it remained relatively high when compared to the healthy plants. At Day 8 the increase was 63% ($P < 0.001$) and 39% ($P < 0.01$) respectively, with respect to the control. At the same time the amino acid content continued to increase in the yellow cultivar but at a lower rate. At Day 8 the increase was 275% of the control ($P << 0.001$). When cultivars were compared among themselves, significant differences were observed among all the cultivars except between healthy plants of the white and yellow cultivars (Table 2). A negative significant correlation ($r_p = 0.940$; $P < 0.05$) between the level of amino acids and the root rot index was found only in the yellow cultivar (Table 3).

Among the amino acids, the red cultivar contained the highest level of constitutive proline (24.0µg g^{-1} fwt), followed by the white cultivar (19.4µg g^{-1} fwt) and lastly by the yellow cultivar (1.0µg g^{-1} fwt). Following inoculation, proline content in the three cultivars decreased gradually, such that at Day 8 it represented 73% and 34% of the control ($P < 0.001$) in the white and red cultivars, respectively (Table 1). It was no longer detected in the yellow cultivar two days after inoculation. Comparison among cultivars at Day 8 showed that differences were significant only between healthy plants (Table 2). A significant positive correlation was found between proline content and the disease in all the cultivars (Table 3).

Phenolics

Constitutively, phenol levels were 0.9µg, 1.0µg and 0.8µg chlorogenic acid g^{-1} fwt in the white, red and yellow cultivars, respectively (Table 1). They remained almost constant during the experiment. Following inoculation, the phenol levels increased progressively and at Day 8 they

Table 1: Level (mean ± SE) of total soluble carbohydrates, amino acids, proline, phenols and lignin in roots of three *Xanthosoma sagittifolium* cultivars inoculated with a zoospore suspension (10^6 ml^{-1}) of *Pythium myriotylum*. In brackets: level of significance of the differences with reference to the control

Time (days)	Soluble carbohydrates (µg glucose mg^{-1} fwt)	Amino acids (µg glycine mg^{-1} fwt)	Proline (µg g^{-1} fwt)	Phenols (µg chlorogenic acid g^{-1} fwt)	Lignins (% of dwt)
White					
0	3.2 ± 0.3 (–)	1.8 ± 0.3 (–)	19.4 ± 1.2 (–)	0.9 ± 0.1 (–)	0.2 ± 0.0 (–)
2	1.6 ± 0.2 (*)	4.3 ± 0.4 (***)	15.8 ± 1.2 (ns)	0.9 ± 0.1 (ns)	0.2 ± 0.0 (*)
4	1.5 ± 0.4 (**)	3.3 ± 0.2 (***)	10.5 ± 0.9 (***)	0.8 ± 0.1 (ns)	0.3 ± 0.0 (**)
6	1.4 ± 0.3 (***)	2.7 ± 0.1 (***)	9.4 ± 1.4 (***)	1.1 ± 0.1 (*)	0.3 ± 0.0 (**)
8	1.3 ± 0.2 (***)	2.7 ± 0.2 (***)	9.0 ± 1.5 (***)	1.2 ± 0.1 (**)	0.3 ± 0.0 (***)
Red					
0	10.1 ± 0.8 (–)	2.5 ± 0.3 (–)	24.0 ± 1.4 (–)	1.0 ± 0.0 (–)	0.2 ± 0.0 (–)
2	6.6 ± 0.8 (**)	5.5 ± 0.4 (***)	20.1 ± 0.8 (*)	1.3 ± 0.1 (ns)	0.2 ± 0.0 (*)
4	4.8 ± 0.7 (***)	4.5 ± 0.3 (***)	13.7 ± 0.7 (****)	2.2 ± 0.1 (****)	0.3 ± 0.0 (***)
6	2.8 ± 0.6 (****)	3.4 ± 0.4 (***)	8.5 ± 1.1 (****)	2.6 ± 0.1 (****)	0.3 ± 0.0 (****)
8	2.6 ± 0.9 (***)	3.4 ± 0.2 (***)	8.0 ± 1.2 (****)	2.7 ± 0.3 (****)	0.3 ± 0.0 (****)
Yellow					
0	10.5 ± 0.7 (–)	1.4 ± 0.3 (–)	1.0 ± 0.8 (–)	0.7 ± 0.0 (–)	0.2 ± 0.0 (–)
2	3.5 ± 0.9 (***)	3.5 ± 0.2 (****)	nd	1.6 ± 0.1 (*)	0.3 ± 0.0 (*)
4	3.4 ± 0.8 (****)	3.6 ± 0.4 (****)	nd	1.7 ± 0.2 (ns)	0.3 ± 0.0 (***)
6	3.3 ± 0.8 (***)	3.7 ± 0.2 (****)	nd	2.3 ± 0.1 (***)	0.4 ± 0.0 (****)
8	2.3 ± 0.9 (***)	4.3 ± 0.2 (****)	nd	3.5 ± 0.1 (***)	0.4 ± 0.0 (****)

nd: not detected

Level of significance: ****P << 0.001; *** P < 0.001; ** P < 0.01; * P < 0.05; ns = not significant; – = not determined

represented 146% (P < 0.01), 272% (P << 0.001) and 198% (P < 0.01) of the control, respectively (Table 1). At Day 8 phenol levels were not significantly different in the healthy plants of white and red cultivars, as was also the case in the white and yellow cultivars (Table 2). A significant correlation (r_p = 0.951 and 0.890; P < 0.05) between the level of phenols and the root rot index was found in the red and yellow cultivars, respectively (Table 3).

The pattern of lignin change was the same as that of phenols. The amount of lignin remained almost constant in the healthy plants (about 0.2% of dwt for the three cultivars) (Table 1). Following inoculation, lignin content increased gradually, and at Day 8 it was 127% (P < 0.001), 159% (P << 0.001) and 186% (P << 0.001) of the control for the white, the red and the yellow cultivars, respectively (Table 1). At Day 8, significant differences (P < 0.05) were observed only between healthy plants of white and red cultivars. Among inoculated plants significant differences were observed between white and yellow cultivars (P << 0.001) and between red and yellow cultivars (P < 0.001) (Table 2). A negative significant correlation (r_p = 0.923; P < 0.05) between the level of lignin and the disease was found only in the yellow cultivar (Table 3).

Qualitative analyses of phenols revealed that the inoculation was characterised by an increase of phenolics in the three cultivars, confirming our quantitative results. Phenolics of the flavanol group (λ_{max} = 220nm) were detected in the three cultivars. Their levels increased with

Table 2: Analysis of variance of the amount of soluble carbohydrates, soluble amino acids, proline, phenols and lignin among three cultivars of *Xanthosoma sagittifolium* eight days after inoculation with a zoospore suspension (10^6 ml^{-1}) of *Pythium myriotylum*. Figures in brackets represent F-values

	Soluble carbohydrates	Amino acids	Proline	Phenols	Lignins
White/Red					
Healthy	**** (195.6)	* (16.6)	* (17.9)	ns (4.6)	* (12.0)
Infected	ns (6.2)	* (18.9)	ns (0.8)	*** (85.9)	–
White/Yellow					
Healthy	na (275.6)	ns (2.2)	na (488.3)	ns (7.8)	–
Infected	ns (1.9)	*** (92.4)	–	na (984,6)	na (507.0)
Red/Yellow					
Healthy	ns (0.4)	** (30.8)	na (578.6)	* (15.7)	–
Infected	ns (0.1)	** (27.7)	–	*** (75.6)	**** (192.0)

–: not calculated

Level of significance: na = not available; ****P << 0.001; ***P < 0.001; **P < 0.01; *P < 0.05; ns = not significant

Table 3: Pearson correlation coefficient analysis between various metabolites and the root rot index (RRI) in three *Xanthosoma sagittifolium* cultivars inoculated with *Pythium myriotylum*. Correlation coefficients were calculated from root rot index (cf. Figure 1) and metabolite content (cf. Table 1) at Days 0, 4 and 8

	Sugar	Amino acids	Proline	Phenols	Lignins	RRI
White						
Sugar	1.0					
Amino acids	0.875	1.0				
Proline	–0.999	0.856	1.0			
Phenols	0.368	0.127		0.403		1.0
Lignins		0.995	–0.918	0.991	–0.277	1.0
RRI	–0.846	0.483	–0.866	0.403	0.791	1.0
Red						
Sugar	1.0					
Amino acids	0.137	1.0				
Proline	–0.978**	–0.046	1.0			
Phenols	0.960**	0.072	0.995**	1.0		
Lignins	0.872	0.110	0.929	–0.960**	1.0	
RRI	–0.933*	–0.131	–0.967**	0.951*	0.836	1.0
Yellow						
Sugar	1.0					
Amino acids	0.990**	1.0				
Proline	–0.990*	0.960**	1.0			
Phenols	0.777	–0.857	0.682	1.0		
Lignins	0.852	–0.891*	0.802	–0.908*	1.0	
RRI	–0.906*	0.940*	0.858	0.890*	0.923*	1.0

Level of significance: ** P < 0.01; * P < 0.05

Table 4: Alterations in amino acid composition and phenol contents in *Xanthosoma sagittifolium* roots eight days after inoculation with a zoospore suspension (10^6 ml^{-1}) of *Pythium myriotylum*

	White		Red		Yellow	
Metabolites	Healthy	Infected	Healthy	Infected	Healthy	Infected
No. of amino acids	5	1	5	3	3	1
Flavanols (λ_{max} = 220nm)	+	++	+	++	+	++
Non-categorised phenols (λ_{max} = 270nm)	+	+	+	++	+	++
Hydroxycinnamic group (λ_{max} = 350nm)	–	–	–	+	–	+

++ = high; + = low; – = not detected

the duration of inoculation. A non-categorised group of phenols (λ_{max} = 270nm) was detected in all cultivars and was influenced by inoculation only in the red and yellow cultivars. Phenolics of the hydroxycinnamic group (λ_{max} = 350nm) were detected in the red and the yellow cultivars after inoculation (data not shown).

Discussion

Some significant qualitative and quantitative alterations, which could be correlated to root rot disease, were recorded among the groups of chemical constituents analysed, following cocoyam inoculation with *P. myriotylum* (Table 4). In inoculated roots, total sugars decreased during the course of our experiments. The decrease was more pronounced in the yellow cultivar than in the white and red cultivars. According to Jeun and Hwang (1991), carbohydrates of the host tissues can be used as carbon substrate by the pathogen. However, the metabolic pathways of sugars and phenols are interrelated (Omokolo *et al*. 1995a). Sugars are major constituents of glycoproteins rich in glycine, proline, and hydroxyproline, that are important constituents of the cell wall. In addition, it is well documented that hydroxyproline, proline and glycine-rich glycoproteins are implicated in plant response against stress (Cassab 1998).

Our results indicate an increase in amino acid content in inoculated roots of all three cultivars, and the dominant amino acid after inoculation appears to be glycine. The increase in amino acids after inoculation could serve in the synthesis of molecules like proteins, phenolics and phytoalexins, which are implicated in plant defence mechanisms (Graham *et al.* 1990, McLusky *et al.* 1999). The disappearance of some amino acids after inoculation suggests that the metabolic pathways of the synthesis of amino acids after plant inoculation are preferentially oriented towards the synthesis of glycine. The work of Hassan *et al.* (1994) on *Lycopersicon esculentum*/*Meloidogyne incognita* interaction, and of Tyuterev and Tarlakovskii (1994) on the dynamics of accumulation of amino acids in wheat under the influence of disease inducers, has shown that some amino acids such as proline, hydroxyproline, glycine and phenylalanine have anti-stress and fungi-toxic properties. In citrus blight disease caused by *Fusarium solani*, Nemec (1995) noted a three-fold increase in soluble amino acids, when compared to healthy trees; asparagine, glutamic acid, proline, glycine and arginine were the most abundant amino acids. Analyses of proline content showed no relationship between its level in the root and the sensitivity of the cultivar, although the accumulation of free amino acids, especially proline, has been associated with stress conditions (Sanchez *et al.* 1996, Omokolo *et al.* 2002). Our finding corresponds with the work of Petrusa and Winicov (1997), who suggested that it is not possible to predict the degree of tolerance of a plant to a given pest from its proline content. The observed decrease in proline content after root inoculation is probably due to an imbalance in their synthesis (Verbruggen *et al.* 1996).

Infection induced a quantitative increase in total phenolic compounds in cocoyam roots. Although the increase was at a lower extent in the white cultivar, this tendency did not seem to be correlated to the degree of resistance of the different cultivars. Other biochemical changes including increases in the activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in inoculated cocoyam roots (unpubl. results) imply the capacity of the plant to stimulate its phenylpropanoid pathway. Many authors have reported the accumulation of phenolic compounds after infection. Omokolo *et al.* (1996) noted a stimulation of the synthesis of phenolic compounds in *Theobroma cacao* after infection by *Phytophthora megakarya*. Cherif *et al.* (1994) observed a pre-existing accumulation of native phenols in the roots of *Cucumis sativus* after infection by *Pythium aphornidermatum* and *Pythium ultimum*. However, it appears that resistance is not always correlated to the quantity of phenols in the tissues, but rather to their nature (Omokolo *et al.* 1996, Daayf *et al.* 1997). In our experiments, preliminary results on qualitative assays have shown an increase in total phenolics in the three cultivars studied, following inoculation. However, their nature differed from one cultivar to the other. Phenols of the flavanol group (λ_{max} = 220nm) and a non-categorised group of phenols (λ_{max} = 270nm) were detected in all cultivars. However, a non-categorised group of phenols was influenced by inoculation in the red and yellow cultivars. Phenols of the hydroxycinnamic group (λ_{max} = 350nm) were detected in the inoculated roots of red (tolerant) and yellow (resistant) cultivars. Flavanols have been shown to have antimicrobial properties (Wink 1988), while phenols of the hydroxycinnamic group play an important role in cell wall rigidification. In fact, they undergo dimerisation through peroxidase-

mediated oxidative coupling. The dimer products cross-link matrix polysaccharides in the cell wall (Sanchez *et al.* 1996). Messner and Boll (1993) have shown that wall extracts containing high levels of diferulic acid bonds are particularly resistant to enzymatic digestion.

The amount of lignin also increased in all the cultivars after inoculation, indicating that the fungal attack constituted a lignifying stimulus. Lignins, by increasing the mechanical strength of cell walls, render them less sensitive to fungal enzymes (Holappa and Blum 1991, Sanchez *et al.* 1996). In addition, they are major constituents of necrotic areas, the formation of which around the point of penetration of the pathogen block the growth and the expansion of the parasite in the plant tissues (Siranidou *et al.* 2002). They also reduce the diffusion of the pathogen toxins (Ride and Barber 1997).

The results of this study show that following inoculation, there were chemical alterations common to all three cultivars. The amount of total soluble sugars and proline decreased, probably due to processes occurring as a result of disease development. Amino acids, phenols and lignin increased in the three cultivars, indicating their involvement in the defence strategy of *X. sagittifolium* to *P. myriotylum*. However, for amino acids, the increase was significantly correlated to the disease (r_p = 0.940, $P < 0.05$) only in the yellow cultivar, whereas for phenols and lignin, there was a correlation in the red and yellow cultivars (r_p = 0.951 and r_p = 0.890, respectively; $P < 0.05$). Preliminary results on qualitative analyses of amino acids and phenols revealed that the accumulation of glycine and flavanol-like phenols was also common to all the cultivars. Besides these common reactions, the accumulation of phenols of the hydroxycinnamic group, whose role in plant resistance is well documented (Assabgui *et al*. 1993, Omokolo *et al.* 1996), was specific to the tolerant red and the resistant yellow cultivars. However, to definitively conclude that these compounds were involved in the resistance response of *X. sagittifolium* would require further analysis and identification using techniques such as TLC and HPLC. It would also be of interest to assess whether amino acid and phenolic synthesis is a key factor of resistance of *X. sagittifolium* under field conditions, since natural infection generally occurs in association with other fungi such as *Fusarium solani* and *Rizotocnia solani*.

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